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Full Length Research Paper

Assessment of genetic variation of wild rohu *Labeo rohita* (Hamilton 1822) populations using microsatellite markers

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For genetic improvement and management of stocks of the Indian major carp, *Labeo rohita*, assessment of genetic variation of wild stocks is essential. Six dinucleotide microsatellite loci *Lr3, Lr12, Lr14b, Lr21, Lr24* and *Lr26* were analyzed to test the genetic variability of three populations of *L. rohita* collected from three different rivers, the Halda, the Jamuna and the Padma, and the analyzed loci were found to be polymorphic (P_{95}) in all the populations. The average number of allele was highest (4.16) in the Jamuna population followed by the Halda (3.66) and the Padma populations (3.16). The average observed heterozygosity (0.633) was highest in the Padma population while the highest average expected heterozygosity (0.607) was obtained in the Jamuna population. Relatively low level of F_{ST} (0.034) and high level of N_m (7.043) was detected between the Jamuna and the Padma populations, while high level of F_{ST} (0.068) and low level of N_m (3.417) was detected between the Halda and the Padma populations. The Nei's (1972) genetic distance (*D*) between the Halda and the Padma populations was the highest (0.167) while that was the lowest (0.073) between the Jamuna and the Padma populations. The results indicated a relatively low level of genetic variation among the wild stocks of *L. rohita* and suggested that necessary measures need to be taken for its stock improvement and conservation.

Key words: *Labeo rohita,* wild population, genetic variation, microsatellite, conservation.

INTRODUCTION

Rohu, *Labeo rohita*, is one of the important Indian major carps (IMCs) in Bangladesh and in South Asia (BBS, 2010). It is a prime aquaculture species and lives in ponds, *haors, baors, beels*, ditches, canals and rivers. It contributes nearly 24.8% to the total aquaculture production in Bangladesh (DoF, 2012). Rohu is a very delicious and lucrative fish and fetches high market price (Rahman, 2005). The natural production of rohu has been decreased due to less recruitment. Natural breeding of rohu has become uncertain due to continuous degradation of spawning and feeding grounds through environmental and human interventions. However, river-originated fry have much better growth performance than hatchery produced fry (Shah and Biswas, 2004).

Earlier seeds of rohu were collected from rivers but the natural seed production has been reduced over the years and its contribution became less than one percent in recent time. Around 1000 hatcheries have been established in private and public sectors and produced required amount of seed from captive reared stocks but maintenance of seed quality is ignored. Inbreeding is a common scenario in private hatcheries, and inter-specific hybrids are produced intentionally or unintentionally in many carp hatcheries (Simonsen et al., 2005). The mass stocking of hybrid fry into open water bodies may cause feral gene introgression into the pure wild stocks. Rohu and other IMCs are prone to loss of genetic purity and thus conservation of genetically distinct wild populations is needed for replenishment of hatchery stocks. Assessment of genetic variation of *L. rohita* is, therefore, essential for conservation and protection of genetic resources.

Genetic variation is referred to the differences in the genetic constituents of the individuals of a species which is necessary to maintain the developmental stability and

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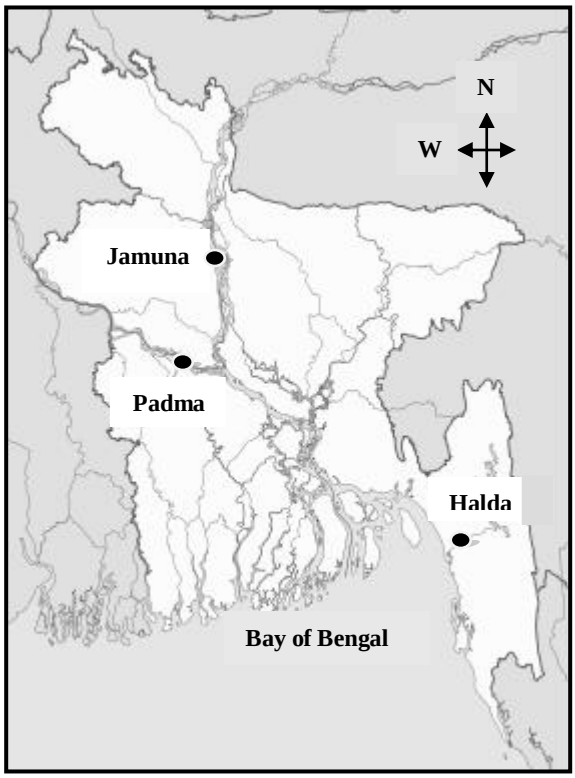

Figure 1. A map of Bangladesh showing sampling sites of *L. rohita*.

biological potential of fish stock (Oster et al., 1988; Tave, 1999; Chauhan and Rajiv, 2010). DNA marker such as RAPD, RFLP, AFLP, microsatellite etc. can be employed for identification of pure stocks, conservation of piscine gene pool, and detection of the genetic changes in both wild and cultured fish species (Liu and Cordes, 2004). Microsatellite is a widely used technique of assessing genetic variation and characterization. It is a short tandemly repeated sequence motif consisting of repeat units of 1-6 bp in length (Tautz and Schlotterer, 1994). Simple sequence repeats (SSRs), termed microsatellite, have been used as genetic markers in many species because the difference in the number of repeats can be easily detected via PCR (Moore et al., 1991). It is widely used for determination of genetic variation in wild and cultured populations (Was and Wenne, 2002). Das et al. (2005) developed twelve microsatellite markers for *L. rohita* and studied polymorphism of these markers in farm reared rohu in India. Alam et al. (2009) analyzed four dinucleotide microsatellite loci (*Lr3*, *Lr12*, *Lr14b* and *Lr21*) to reveal population genetic structure of *L. rohita* in Bangladesh. Saha et al. (2010) assessed the population genetic structure of an endangered carp, *Labeo calbasu*, collected from three different stocks; the Jamuna river, the Halda river and a hatchery of Bangladesh using four heterologous microsatellite loci (*Lr12*, *Lr14b*, *Lr21, Lr24*) identified from *L. rohita*. Since, natural recruitment of rohu has been seriously hampered over the years due to environmental and anthropogenic interferences, assessment of genetic variation of wild *L. rohita* populations using microsatellite markers is crucial for production and conservation of this species. The present study was, therefore, designed to assess the genetic variation of three riverine, the Padma, the Jamuna and the Halda rohu stocks using six dinucleotide microsatellite loci *Lr3*, *Lr12*, *Lr14b*, *Lr21, Lr24* and *Lr26* with an expectation that the findings would be useful to formulate strategies for genetic management and conservation of *L. rohita*.

MATERIALS AND METHODS

Collection of Fish Samples

Fry of *L. rohita* were collected from three different rivers; the Halda (Modonaghat, Chittagong), the Jamuna (Jamalpur) and the Padma (Ishardhi, Pabna) in 2010 and reared separately in ponds located beside the Faculty of Fisheries, Bangladesh Agricultural University, Mymensingh. Fry were reared primarily with soaked mustard oil cake and then commercial supplemental feed (Mega Feed-nursery) for 45 days. Then the fingerlings were stocked in brood rearing ponds and fed with commercial supplemental feed (Mega Feed-pellet) until getting maturity. A total of 90 fish samples, 30 from each of three rivers were collected for the study. The locations of the sampling sites are shown in Figure 1. The fish were anesthetized with MS 222, and approximately 30 mg of caudal fin tissue was clipped from each individual using a sterile scissor.

Isolation of Genomic DNA

The collected fin samples were cut into small pieces with a pair of scissors and ground with a tissue grinder in a 1.5 ml eppendorf tube. The genomic DNA was extracted through phenol: chloroform: isoamyl alcohol (25:24:1 v/v/v) extraction, and ethanol precipitation method as described by (Islam and Alam, 2004).

Primer Selection and Amplification of Microsatellite Loci

Six primer pairs for *L. rohita* microsatellite markers *Lr3, Lr12, Lr14b, Lr21, Lr24* and *Lr26* developed by Das et al. (2005) were used in this study. For PCR amplification, 50 ng of DNA was used as template for 12 µl reaction mixture containing 0.25 mM each of the dNTPs, 2.0 µM of each primer, 1.5 mM $MgCl_2$, 1 µl 10X reaction buffer and 1 unit of Taq DNA polymerase. Initial template denaturation was accomplished at 94ºC for 3 min followed by 35 cycles of 30 sec at 94ºC for denaturation, 30 sec at 57° C for annealing and 1 min at 72ºC for extension. The final step

Table 1. Frequencies of alleles at six microsatellite loci in three populations of *L. rohita*

Locus	Allele size	Halda	Jamuna	Padma
Lr3	131	0.2000	0.1000	0.0250
	134	0.3250	0.3250	0.2250
	137	0.4750	0.2000	0.3250
	139	0.0000	0.3750	0.4250
Lr21	143	0.0000	0.1000	0.0000
	148	0.8000	0.5500	0.7000
	159	0.1500	0.2500	0.2500
	163	0.0500	0.1000	0.0500
Lr12	156	0.0000	0.2250	0.0000
	163	0.2500	0.3750	0.7000
	165	0.4750	0.2000	0.3000
	170	0.2000	0.0750	0.0000
	172	0.0750	0.1250	0.0000
Lr14b	171	0.1750	0.1250	0.1250
	174	0.2000	0.2250	0.5000
	177	0.5750	0.5750	0.2500
	188	0.0500	0.0750	0.1250
Lr24	150	0.1000	0.0750	0.0000
	154	0.0250	0.0500	0.2000
	157	0.5000	0.0500	0.0000
	160	0.1750	0.5500	0.5250
	165	0.1750	0.2250	0.2250
	167	0.0250	0.0500	0.0500
Lr26	144	0.2500	0.1500	0.1750
	146	0.7500	0.8500	0.8250
No. of missed allele across loci		3	0	6

was extended for 5 min at 72ºC. When the PCR was completed, the PCR products were kept in a refrigerator (4ºC) for electrophoresis.

PCR was performed in a 12-µl reaction volume containing 50 ng of template DNA, 2.0 µM of each primer, 0.25 mM each of the dNTPs, 1 unit of Taq DNA polymerase, 1.5 mM $MgCl_2$ and 1 µl 10X reaction buffer. The temperature profile consisted of 3 min initial denaturation at 94ºC followed by 35 cycles of 30 sec at 94ºC for denaturation, 30 sec at 57° C for annealing, and ending with 5 min at 72ºC for final extension. When the PCR was completed, the PCR products were kept in a refrigerator (4ºC) for electrophoresis.

Electrophoresis and visualization of PCR products

PCR products (6 µl) were confirmed by running through a 2% agarose gel containing 3µl ethidium bromide in 1×TBE buffer. DNA bands were observed under UV light on a transilluminator and photographed by a Gel Doc with digital camera. Following confirmation of PCR amplification, 3µl of PCR product of each sample were electrophoresed on a 6% denaturing polyacrylamide gel containing 19: 1 acrylamide : bis-acrylamide and 7M urea. The electrophoresis was accomplished by using the SequiGen GT sequencing gel electrophoresis system (Bio-Rad Laboratories, Hercules, CA). After pre-heating, the PCR products were loaded between the teeth of the comb, and the DNA ladder was loaded on either side of the gel. The gel was run with the power set at 60 W and temperature set at 50°C for required length of time (1h 20 min) according to the size of the DNA fragment. After completion of electrophoresis, the gel was stained with silver nitrate following Promega (Madison, WI) silver staining protocols for visualization of the DNA fragments.

Scoring and statistical analysis of microsatellite data

The electrophoretic bands corresponding to particular allele at each locus were alphabetically named as a, b, c etc. in order of detection from the top to the bottom of the gel. Allele frequencies were calculated directly from observed genotypes. A single genotypic data matrix was prepared for all loci. The marker and allelic length was measured by the software DNA frag version 3.03 (Nash, 1991). The software GenAlEx version 6.1 (Peak all and Smouse, 2005) was used for estimating the number of alleles (N), and frequency of alleles. Allelic variation and fit to Hardy-Weinberg proportion were estimated using the software POPGENE (version 1.31) (Yeh et al., 1999) by a chi-square (X^2) test with 1000 simulated samples. Expected (H_e) and observed heterozygosity (H_o), pairwise homogeneity test, genetic differentiation (F_{ST}), gene flow (*Nm*) and the genetic distance values (*D*) between

Table 2. Allelic and genetic variation at six microsatellite loci in three populations of *L. rohita* (*N* =No. of alleles, H_o =heterozygosity observed, H_e =heterozygosity expected).

Microsatellite loci	Parmeters	Halda	Jamuna	Padma
Lr3	*N(4)*	3	4	4
	H_O	0.9500	0.9500	1.0000
	H_e	0.6449	0.7218	0.6795
	1-H_O / H_e	-0.47	-0.31	-0.49
Lr21	*N(4)*	3	4	3
	H_O	0.3000	0.6000	0.6000
	H_e	0.3436	0.6308	0.4564
	1-H_O / H_e	0.12	0.05	-0.31
Lr12	*N(4)*	4	5	2
	H_O	0.5500	0.6000	0.5000
	H_e	0.6833	0.7667	0.4308
	1-H_O / H_e	0.20	0.22	-0.16
Lr14b	*N(4)*	4	4	4
	H_O	0.6000	0.7000	0.5500
	H_e	0.6115	0.6128	0.6731
	1-H_O / H_e	0.01	-0.15	0.18
Lr24	*N(4)*	6	6	4
	H_O	0.6500	0.4000	0.8000
	H_e	0.6949	0.6500	0.6474
	1-H_O / H_e	0.06	0.385	-0.25
Lr26	*N(4)*	2	2	2
	H_O	0.5000	0.3000	0.3500
	H_e	0.3846	0.2615	0.2962
	1-H_O / H_e	-0.31	-0.15	-0.20
Average H_o over loci		0.5917	0.5917	0.6333
Average H_e over loci		0.5605	0.6073	0.5306
Average number of alleles		3.66	4.16	3.16
Polymorphism (P_{95})		1.00	1.00	1.00

the population pairs were also calculated with the help of POPGENE (version 1.31) (Yeh et al., 1999) computer package program.

RESULTS

Allelic and genotypic variations between populations

Six microsatellite loci *Lr3, Lr21, Lr12, Lr14b, Lr24, Lr26* analysed for the wild populations of rohu (the Halda, Jamuna, Padma) were found to be polymorphic (P_{95}) and the number of allele across the loci was 2 to 6. The sizes of the alleles for all loci in three populations ranged from 131 to 188bp. The Jamuna population possessed two private alleles, $Lr21_{143bp}$ and $Lr12_{156bp}$ which were absent in two other populations (Halda and Padma) (Table 1). The Halda population had a missing of three alleles ($Lr3_{139bp}$, $Lr21_{143bp,}$ $Lr12_{156bp}$) while six alleles were missed in Padma population ($Lr21_{143bp}$, $Lr12_{156bp}$, $Lr12_{170bp}$, $Lr12_{172bp}$, $Lr24_{150bp,}$ $Lr24_{157bp}$) (Table 1). The locus *Lr24* in the Halda and the Jamuna populations had the highest 6 alleles while the locus *Lr26* had the least 2 alleles in all three populations (Table 1). The average number of alleles, 4.16 was the highest in the Jamuna population and that of 3.16 was the lowest in the Padma population. The Halda population contained the average number of alleles, 3.66. The observed average heterozy

Table 3. Deviation from Hardy–Weinberg genotype frequency expectations in three different populations of *L. rohita* (χ^2 values, followed by degrees of freedom in parentheses).

Parameters	Loci	Halda	Jamuna	Padma
H-W test	*Lr3*	17.31*** (3)	13.13* (6)	14.47* (6)
	Lr21	11.66** (3)	29.52*** (6)	3.31 ^{ns} (3)
	Lr12	9.46 ^{ns} (6)	28.91** (10)	0.55 ^{ns} (1)
	Lr14b	9.00 ^{ns} (6)	3.90 ^{ns} (6)	39.54 *** (6)
	Lr24	10.97 ^{ns} (15)	74.03*** (15)	8.13 ^{ns} (6)
	Lr26	1.96 ^{ns} (1)	0.50 ^{ns} (1)	0.75 ^{ns} (1)

Statistically significant values are marked with asterisks. NS=not significant, *P <0.05, ** P<0.01, *** P<0.001.

Table 4. Homogeneity between the samples of *L. rohita* (X^2 values followed by degrees of freedom in parentheses).

Loci	Stocks	Jamuna	Padma
Lr3	Halda	6.85^{ns} (3)	1.16 ^{ns} (3)
Lr21	Halda	7.51*(3)	1.26^{ns}(2)
Lr12	Halda	17.25***(4)	21.10***(3)
Lr14b	Halda	0.41^{ns}(3)	11.17**(3)
Lr24	Halda	23.54***(5)	37.02***(5)
Lr26	Halda	1.25 ^{ns} (1)	0.67 ^{ns} (1)
Lr3	Jamuna		3.84 ^{ns}(3)
Lr21	Jamuna		5.38 ^{ns}(3)
Lr12	Jamuna		21.73***(4)
Lr14b	Jamuna		10.88**(3)
Lr24	Jamuna		8.62 ^{ns}(3)
Lr26	Jamuna		0.091 ^{ns}(1)

Statistically significant values are marked with asterisks. NS=not significant,*P<0.05, ** P<0.01, *** P<0.001

gosity (H_o) value in the Padma stock was the highest (0.63) followed by the Halda (0.59) and the Jamuna population (0.59) (Table 2). The analysis of 1-H_o/H_e values in 10 out of 18 cases were found negative and it indicated that all the three populations were excess in heterozygosity at those loci. On the other hand, the remaining eight 1-H_o/H_e values were positive and indicated the deficiency in heterozygosity (Table 2). The results of Hardy-Weinberg Equilibrium (HWE) for all the six loci showed significant deviation from equilibrium in 8 out of 18 tests. The Halda and Padma populations got deviation from HWE at two loci each while the Jamuna population deviated at four loci (Table 3).

Inter population genetic structure and pair-wise comparisons of different stocks using homogeneity tests are presented in Table 4 and eight out of eighteen tests were significant. This analysis revealed significant differences between the Halda and the Jamuna stocks at loci *Lr21, Lr12* and *Lr24*, between the Halda and the Padma stocks at loci *Lr12, Lr14b* and *Lr24,* and between the Jamuna and the Padma stocks at loci *Lr12* and *Lr14b* (P<0.05, P<0.01, P<0.001). The population differentiation (F_{ST}) value between the Halda and the Padma population was the highest (0.068) while that was the lowest (0.034) between the Jamuna and the Padma population. The gene flow was highest between the Jamuna and the Padma population (7.043) and lowest between the Halda and the Padma population (3.417) (Table 5).

Genetic distance between three wild populations was estimated and highest genetic distance was found between the Halda and the Padma population (0.167). The lowest genetic distance was existed between the Jamuna and the Padma population (0.073) (Table 6). The UPGMA dendrogram based on Nei's (1972) genetic distance

showed that the three populations were laid in two major clusters. The Halda population was in one cluster and the remaining two populations (the Jamuna and the Padma) were in other cluster (Figure 2). The dendrogram showed relatively more distance between the Halda and the Padma populations.

DISCUSSION

L. rohita is a prime aquaculture species in Bangladesh and farmers were used to collect seed from different ri-

Table 5. Multilocus N_m and F_{ST} values between pairs of three populations of *L. rohita* across all loci.

Population	Pair-wise F_{ST}	Mean F_{ST}	Pair-wise N_m	Mean N_m
Halda-Jamuna	0.046		5.117	
Halda-Padma	0.068	0.065	3.417	3.593
Jamuna-Padma	0.034		7.043	

Table 6. Nei's unbiased measures of genetic identity (above diagonal) and genetic distance (below diagonal) in three different populations of *L. Rohita.*

Population	Halda	Jamuna	Padma
Halda	****	0.887	0.846
Jamuna	0.120	****	0.929
Padma	0.167	0.073	****

vers for aquaculture. The supply of naturally produced seed has been reduced due to environmental and anthropogenic activities and the genetic constitution of the species was also changed. All the hatcheries are producing seeds from domesticated stocks of rohu but wild stocks need to replenish the captive stocks for quality improvement. The genetic structure of the wild population of rohu is assumed to be changed over the years but very little investigation was done in this regard. Previously Islam and Alam (2004) and Khan et al. (2006) analysed the genetic structure of rohu from the Halda, Jamuna and Padma rivers using RAPD and allozymes markers and found 29% and 27% polymorphic loci across the three populations respectively. Alam et al. (2009) genetically characterized the three wild populations of rohu collected from the Halda, Jamuna and Padma rivers in 2006 using four microsatellites markers and found little genetic variation among the populations. After five years of their study seed of rohu were collected from the same three rivers but from different locations and samples were genetically characterized using six microsatellite markers including the four markers used by Alam et al. (2009). Though two studies were conducted with the fish originated from same river systems, a comparative statement can be made using the differential results for the stocks with time and space.

Genetic variability is fundamental to ensure the evolutionary potential of populations (Frankel and Soule, 1981; Koljonen et al., 2002). Loss of genetic variation is considered to be the factor for loss of genetic potential of fish stock and adaptation to environmental changes. Luikart et al. (1998) suggested that inadequate management of natural populations could cause genetic erosion and lead to population and even species extinction. For detecting genetic variability in *L. rohita* populations in the present study, microsatellite markers have been found to be suitable. Six microsatellite loci (*Lr3, Lr21, Lr12, Lr14b, Lr24, Lr26*) that have been tested were found to be polymorphic in all the three populations which were consistent with the findings of Alam et al. (2009) who obtained similar genetic polymorphism in different wild stocks of *L. rohita* by four microsatellite markers. The microsatellite markers used in this study were developed by Das et al. (2005) who obtained all polymorphic loci in an Indian farm-reared population of *L. rohita* through these markers. Similar result was also reported from microsatellite markers of *Cyprinus carpio* (Zhou et al., 2004). The observed heterozygosity as obtained in the present study was higher than that of Alam et al. (2009).

The average number of alleles was the lowest (3.16) in the Padma stock and they have the highest number of null alleles (6.0). On the other hand, the highest average number of alleles (4.16) was found in the Jamuna population and it had no null allele. Loss of allelic variation has been reported from the same three riverine populations of *L. rohita* by Alam et al. (2009) where highest average number of allele was obtained from Jamuna (3.50) and lowest from Halda (2.75). In addition, maximum 3 alleles were missed from Halda stock and 2 from Padma stock. From the two studies it has been observed that average number of alleles increased in the present study and at the same time the number of null alleles was also increased. This might be observed due to use of more microsatellite markers but increase the number of null alleles specially for Padma stock indicated an adverse situation for existence in the wild. However, it is interesting that Jamuna population in both studies showed highest average number of alleles and there was no null allele. Alam and Islam (2005) studied genetic structure of another member of Indian major carps, *Catla catla* collected from the Halda, Padma and Jamuna rivers and from a private hatchery and found similar allelic variation like rohu. Using the same six microsatellite loci Das et al. (2005) obtained a total of 29 alleles in *L. rohita* in India but a total of 25 alleles were obtained from this study. This data indicated that *L. rohita* populations in Bangladesh are in

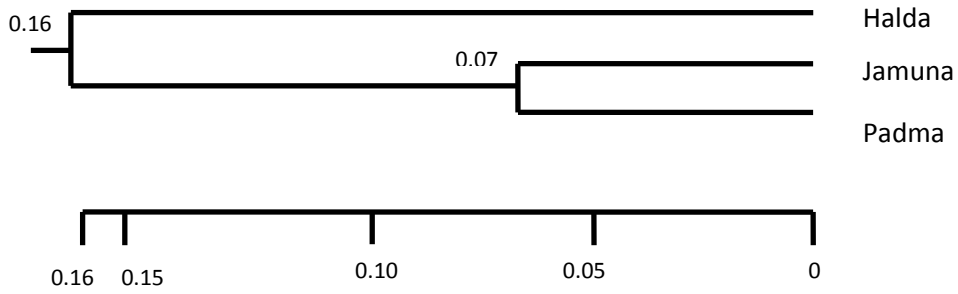

Figure 2. UPGMA dendrogram based on Nei's genetic distance.

stressed condition and have lost a few alleles. If it is happening, more alleles will be lost in future and the number of the null allele will be increased.

A more appropriate measure of genetic variation within a population is gene diversity (average expected heterozygosity). In this study the average observed and expected heterozygosity of three populations ranged from 0.591 to 0.633 and 0.530 to 0.607 which were lower than those values of 0.655 and 0.617 respectively obtained from the same six loci of *L. rohita* (Das et al., 2005). It was evident that the river originated wild populations of rohu have lost genetic variability but the actual reasons were difficult to explain. However, some causes those are being happened most often such as collection of fertilized eggs during the breeding season from Halda river, overfishing of broods and spawn, river siltation, construction of dam and other natural or man-made activities interfere the natural breeding and feeding migration, reduction of natural recruitment are held responsible. Furthermore, mass stocking of open waters with low quality hatchery produced seeds and escaping of hatchery originated fish from culture ponds during flooding in rainy season also affect the genetic diversity and purity of the wild stocks. Reduction of genetic diversity of natural stocks of salmonid species due to hatchery operation was reported by Wang et al. (2002).

In Hardy-Weinberg comparisons, the observed heterozygosity values in 10 out of 18 cases were higher than the expected heterozygosity in the present study whereas previous studies involving wild stocks of *L. rohita* from the same sources demonstrated similar results (5 *Ho* values out of 12 were higher than the *He*) (Alam et al., 2009). Similar findings were also reported from another Indian major carp, *Labeo calbasu* by Saha et al. (2010). The three wild populations of rohu have got positive values of 1-H_o/H_e in four out of six loci but in majority cases they have negative values of 1-H_o/H_e in all the loci. The negative values of 1-H_o/H_e as obtained in all the populations referred that the populations did not loss heterozygosity and individuals could be considered as outbreds. The positive values of 1-H_o/H_e indicated the loss of alleles, and heterozygosity may be decreased with bottlenecking and inbreeding. The high genetic diversity observed in a population could be explained by the overlap of generations, population mixing from different geographical locations, natural selection favouring heterozygosity or subdivision accompanied by genetic drift (Begum et al., 2013).

Among the six microsatellite loci tested the Halda and the Padma populations significantly (P<0.05, P<0. 01, P<0.001) deviated from Hardy-Weinberg proportions at two loci while the Jamuna population significantly (P<0.05, P<0.01, P<0.001) deviated at four loci. The deviation from Hardy-Weinberg equilibrium in the Halda stock at locus *Lr3,* the Jamuna stock at loci *Lr21*and *Lr24* and the Padma stock at locus *Lr14b* was significantly high (P<0.001) while the deviation in the Jamuna and the Padma stocks at locus *Lr3* was significantly low (P<0.05) due to loss of heterozygosity and it might be resulted from habitat degradation and bottle necking. Among the three populations, significant deviations observed in Jamuna population for most of the loci indicating poorest genetic variation in the stock. Islam et al. (2007) suggested that reduction in allelic variation could be explained by founder effect and genetic drift in the population attributed from a sudden decrease in effective population size (N_e).

Analysis of genetic differentiation (F_{ST}) between the three populations of rohu demonstrated a low level of genetic variation with a range of 0.034 to 0.068. The genetic differentiation between the Jamuna-Padma stocks remained lowest and that of highest in Halda-Padma stocks. The pair-wise comparisons of different stocks of *L. rohita* using homogeneity tests revealed that the Jamuna and Padma population were more homogeneous

compared to the Halda-Jamuna and the Halda-Padma population pairs and it supports the findings of above genetic differentiation. Similarly, highest gene flow and lowest genetic distance were observed between Jamuna and Padma populations. Simonsen et al. (2005) found close genetic relationship in catla (*Catla catla*) and rohu (*L. rohita*) samples collected from the Halda, Jamuna and Padma rivers, even though the Halda river is located in eastern part of Bangladesh and geographically isolated from the Jamuna-Padma river system. The geological structures separated the Halda river from the other stocks and might limit the gene flow between the Halda and any

of the other two populations. On the other hand, the Jamuna and the Padma rivers are located in the south-western part of Bangladesh and joined to each other near the Goalanda upazila of Rajbari district so there might be a possibility of intermixing of the fishes of these two inter-connected rivers. Another explanation is that recurrent flooding events could provide corridors for gene flow between the rivers and alternatively, open water fish stocking and escaping of fish from farms could have contributed to homogenization of gene pools.

The analysis of six microsatellite loci of the wild *L. rohita* revealed some degree of intra and inter population genetic variation in three riverine populations but the overall genetic variation was quite low. Similar findings were obtained by Alam et al. (2009) from analysis of four microsatellite loci four years back. Therefore, it is evident that the wild populations of *L. rohita* have been under stress over the years and genetic degradation is taking place day by day that emphasizing maintenance of genetic variation in the stocks through proper management. To increase the genetic variation and restoration of missed alleles the most important strategy is to increase the population size which can be done through stopping over-exploitation of broods and seeds, setting up adequate number of fish sanctuaries, discourage of stocking of poor quality fry in open water bodies, improvement of breeding and feeding grounds by restoration of water flow of rivers, increase of public awareness, and if possible periodic genetic assessment of fish samples from different wild stocks.

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